REMARKS

Claims 23-40 were pending in the application. Claims 27-30 have been cancelled. Claims 23, 24, 35 and 36 have been amended. Support for the amendments to claims 23, 24, 35, and 36 can be found throughout the specification, including at least at page 21, lines 35-36 and page 48, lines 32-33, and at page 51, lines 35-39. Thus, upon entry of the entry of the foregoing amendments, claims 23-26 and 31-40 will be pending.

No new matter has been added. Applicants request that the amendments and cancellation of claims be entered. The foregoing claim amendments and cancellations should in no way be construed as an acquiescence to any of the Examiner's rejections and were made solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Rejection of Claims 23-40 Under 35 U.S.C. §112, First Paragraph

Claims 23-40 have been rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The Examiner states that while the specification is enabling with regard to a transgenic mouse, the specification "does not reasonably provide enablement for all other transgenic organisms embraced by the claims." The Examiner also states that Applicants have "not provided adequate guidance to overcome the unpredictability of a phenotype resulting from expression of a transgene in different species." Applicants respectfully traverse this rejection.

As amended, the claims describe a non-human transgenic animal having a transgene integrated into the genome of the animal, and, in one embodiment, a *tet* operator-linked gene in the genome of the animal. The claimed transgene comprises a transcriptional regulatory element functional in cells of the non-human animal operatively linked to a polynucleotide sequence encoding a fusion protein which activates transcription of a *tet* operator linked gene, wherein the fusion protein comprises a first polypeptide which is a Tet repressor, operatively linked to a second polypeptide which

directly or indirectly activates transcription of a *tet* operator-linked gene at a ***detectable level***.

The Examiner states that "the issue is not whether or not transgenic non-human animals other than mice can be created," but questions "whether expression of a tet operator-linked gene will produce a reproducible phenotype when expressed in different species of transgenic non-human animals." Applicants submit that the "phenotype" to which the Examiner is referring is dependent upon the gene of interest which is operatively linked to the tet operator to which the fusion protein binds. Applicants respectfully point out that the pending claims do not require a phenotype, but rather recite that the transgene activate transcription of a tet operator-linked gene at a ***detectable level***.

Based on the instant specification and methods of making transgenic animals known at the time of filing, Applicants submit that one of ordinary skill in the art would be able to make and use the claimed non-human transgenic animal such that the gene of interest is expressed at detectable levels, as required by the claims. In Example 2 of the specification, Applicants teach transgenic mice comprising the tTA regulatory system, wherein the *tet* operator-linked gene of interest is the luciferase reporter gene. As shown in Figure 14 of the specification, double transgenic mice comprising the tTA fusion protein and the *tet* operator-linked luciferase gene show detectable levels of luciferase in the absence of tetracycline. As required by the claims, transactivation by the claimed fusion protein results in ***detectable levels*** of luciferase reporter gene expression, measured as luciferase activity. Based on the examples provided in the instant specification, Applicants show that the claimed tTA expression system is a predictable system which provides a precise mechanism for controlling expression of a gene of interest in a detectable manner.

The claimed transgenic animal comprises a tTA expression system which allows for transcriptional control of a specific gene of interest such that the transcription is

detectable. The Examiner has equated detectable expression with a resulting phenotype, which are not necessarily equivalent. For example, in Example 2 of the specification, the gene of interest is a reporter gene, wherein the phenotype is luciferase activity. Incidentally, luciferase activity also provides a means of detecting gene expression. In other examples, such as the Ebert *et al.* reference previously cited by the Examiner, gene expression and phenotype are not necessarily equivalent. In the Ebert reference, the authors describe transgenic pigs which express a rat somatotropin (rGH) transgene, resulting in a phenotype of increased skeletal growth. Applicants maintain that the resulting phenotype depends on the nature of the gene of interest and is often the subject of the scientist's hypothesis, wherein the resulting phenotype is unknown until evaluated through the scientific process, exemplified by Ebert and the references cited herein (discussed below). Applicants provide a gene expression system which can be used to create transgenic animals comprising a predictable gene expression system, which in turn allows one of ordinary skill in the art the opportunity to study the phenotype of the transgenic animal.

In maintaining the position that the art of making a transgenic animal with a predictable phenotype was unpredictable at the time of filing, the Examiner alleges that "the evidence of record has not provided adequate guidance to overcome the unpredictability of a phenotype resulting from expression of a transgene in different species of non-human animals."

As the Examiner is aware, the standard regarding predictability in the art refers to "the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art." (see MPEP 2164.03). Applicants submit that one of ordinary skill in the art would recognize that the process of making a transgenic animal may

require the screening of an initial litter of transgenic animals in order to identify founders who express a certain detectable level of the gene of interest, who, in turn, are then used to propagate a transgenic line (see specification at pages 11, lines 12-13; page 50, lines 33-34; and page 51, lines 4-5).

In support of Applicants' assertion regarding the predictability of making transgenic animals comprising gene expression systems resulting in detectable expression of a gene of interest, Applicants provide the following references which describe transgenic animals comprising the tTA or reverse tTA (reverse tTA comprises a mutant version of TetR with reverse binding properties) gene expression systems:

1. Bello *et al.* (1998) *Dev.* 125:2193-2202, describes use of the claimed tTA system in *Drosophila* (enclosed as Appendix A);
2. Girard *et al.* (1998) *Embo J* 17: 2079-85, describes use of the claimed tTA system in *Drosophila*, wherein the synthetic *MATH20* gene is controlled by the tTA system of position effect variegation (enclosed as Appendix B);
3. Heinrich and Scott (2000) *Proc Natl Acad Sci U S A* 97: 8229-8232, describes use of the claimed tTA system in *Drosophila* for making transgenic insect strains suitable for a sterile-release program (enclosed as Appendix C);
4. Horn and Wimmer (2003) *Nat Biotechnol* 21: 64-70, describes a transgene-based, embryo-specific lethality system for insect pest management using the claimed tTA system (enclosed as Appendix D);
5. Bieschke, *et al.* (1998) *Mol Gen Genet* 258:571-579, describes transgenic flies comprising the reverse tTA gene expression system to control transcription of *tet*-operator linked gene *lacZ* (enclosed as Appendix E);
6. Landis *et al.* (2001) *Genetics* 158: 1167-1176, describes use of the reverse tTA system in *Drosophila* in a study examining gene function *in vivo*, wherein some

of the genes are involved in multiple time periods during development. The authors of Landis note in the abstract, the tetracycline gene control system provides an ideal conditional gene expression system which allows for temporal transcriptional control (enclosed as Appendix F);

7. Allikian *et al.* (2002) *Genome Biol* 3: research0021.1–research0021.10, describes use of the tetracycline-regulated gene expression system for controlling RNA interference (RNAi) for the *pgm* gene in a study of aging *Drosophila* (enclosed as Appendix G);
8. Melfi *et al.* (2000) *J. Mol. Biol.* 304(5):753-763, describes use of the claimed tTA expression system in sea urchins to control expression of a *sns* fragment (enclosed as Appendix H);
9. Ridgway, *et al.* (2000) *Exp. Cell Res.* 256(2):392, describes the effectiveness of the claimed tTA gene expression system in *Xenopus* (enclosed as Appendix I);
10. Das and Brown (2004) *Proc.Natl.Acad.Sci.* USA 101:4839-4842, describes use of the claimed tTA gene system in *Xenopus* to express a mutant form of the thyroid hormone receptor (enclosed as Appendix J);
11. Braudeau *et al.* (2003) *Exp Biol Med* 228(5):466-71, describes transgenic rats comprising the claimed tTA gene expression system to control expression of the *heme oxygenase-1* (*HO-1*) gene (enclosed as Appendix K); and

The above-mentioned references describe different transgenic animals or animals containing transgenes, including *Drosophila*, *Xenopus*, sea urchins, and rats. Each of these transgenic animals comprises a transgene encoding a transcriptional activator fusion protein which activates expression of a *tet* operator-linked gene of interest. These references are representative of the claimed non-human transgenic animals comprising the tTA gene expression system, and demonstrate the predictable, successful use of a

transgene comprising a fusion protein which activates transcription of a *tet* operator-linked gene of interest. Given that transcriptional regulatory systems described in the above-mentioned references can function in *Xenopus, Drosophila,* sea urchins, and rats, all of which are phylogenetically diverse organisms, it would be expected, and in fact is proven by the above-mentioned references that the claimed tTA transcriptional regulatory system functions in non-human transgenic animals other than mice. If the Examiner maintains the assertion that making transgenic animals in accordance with the claimed invention is unpredictable, Applicants respectfully request that the Examiner point to evidence to support this assertion and/or explain why each of the submitted references does not mention the predictability of creating a transgenic animal expressing a *tet* operator-linked gene of interest at detectable levels.

Furthermore, the above-mentioned Bello reference speaks to Applicants position regarding detectable gene expression and phenotypes in non-human transgenic animals. The Bello reference describes successful use of the claimed tTA expression system to control transcription of *lacZ* and *ANTP* genes in transgenic flies. The engineered transgenic flies show detectable expression of *tet*-operator linked genes (*lacZ* and *ANTP*), resulting in phenotypes including head and eye-specific defects due to mis-expression of *ANTP*. To show detectable expression of the *tet* operator-linked gene, the authors assay protein levels of the ANTP protein (see Figure 1C). The authors then investigate whether ectopic expression of the *Antp* results in a phenotype, and determine that overexpression of *Antp* causes defective head formation (see Figure 1B). Notably, in flies transgenic for *ey-tTA* and *tetO-ANTP*, wherein the tTA transactivator was under the transcriptional control of an eye-specific promoter, eye-specific defects were not detected in flies fed tetracycline, whose *tet* operator-linked gene was silent. Eye defects were visible, however, in flies with ectopic *ANTP* expression who were not fed tetracycline (see page 2198, top left paragraph). Thus, the authors of the Bello reference make a clear

distinction in their report between detectable expression of the *tet* operator-linked genes and resulting phenotypes.

The Examiner alleges that "Applicants have not addressed why or how the tTA system overcomes the position effect since endogenous transcriptional machinery is needed." Applicants respectfully maintain that at the priority date of the instant application, the teachings set forth in the specification with regard to the general construction of transgenic organisms (see *e.g.*, pages 18-24), was routinely utilized in the production of a variety of transgenic organisms with predictable phenotypes, including rats, pigs, sheep, cows, and other domestic farm animals. Applicants refer the Examiner to the following references which are enclosed herewith, each of which supports the predictability of the presently claimed invention in animals other than mice. In addition, each of the following references also supports the idea that issues surrounding the position effect can be readily overcome by one of ordinary skill in the art and do not impede production of a transgenic non-human animal, as asserted by the Examiner.

Hammer *et al.* (1985) *Nature* 315: 680 (Appendix L – hereinafter "Hammer-1985") describes microinjection of a transgene construct encoding the mouse metallothionein -I (MT) promoter/regulator region fused to the human growth hormone gene (hGH) into pronuclei or nuclei of eggs from superovulated rabbits, sheep and pigs. The authors analyzed the frequency of integration of the transgene in all three experimental species, as well as the expected phenotype of the transgenic rabbits and pigs, *i.e.,* expression of hGH. The data described in Hammer-1985 show that the frequency of integration, equivalent to the number of transgenic offspring, was 12.8% in rabbits, 11% in pigs, and 1.3% in sheep (see Table I). Expression of the transgene revealed that out of sixteen rabbits, four had detectable levels of hGH specific mRNA in the liver. Thus, 25% of the successful transgenic animals exhibited detectable levels of the transgene. For transgenic pigs, eleven out of eighteen transgenic animals expressed

the transgene, corresponding to 61%. Expression in sheep was not determined as only one transgenic sheep was obtained.

Mullins *et al.* (1990) *Nature* 344:541 (Appendix M – hereinafter "Mullins-1990") describes the introduction of the mouse *Ren-2* renin gene into rats and provides an analysis of the resulting phenotype, namely hypertension or elevated blood pressure. The results provided in Mullins-1990 demonstrate an integration frequency of 62.5%, as five out of eight rats carried the transgene. Four of the transgenic founder rats were bred successfully, and, notably, all four transgenic founder rats had elevated blood pressure compared to control animals that did not carry the transgene. Furthermore, analysis of a transgenic line established from a transgenic founder male rat demonstrated that, without exception, progeny inheriting the *Ren-2* renin transgene, also had the hypertensive phenotype.

The techniques described in Hammer-1985 and Mullins-1990 thus resulted in successful production of transgenic animals other than transgenic mice, including transgenic pigs, rabbits, and rats. Both Hammer-1985 and Mullins-1990 show that transgenic animals can be obtained through microinjection of DNA into the pronuclei or nuclei of eggs as taught by the instant specification, which is the standard procedure still today, for generating transgenic animals. These references, therefore, demonstrate the efficacy and predictability of the techniques taught in the instant specification in the production of transgenic animals other than mice, including pigs, rabbits, and rats. In addition, a recent paper, Auerbach *et al.* (2003) *Transgenic Research* 12:59 (enclosed herewith as Appendix N), shows that even today, transgenic mice are obtained at an overall frequency of 15-20% (see Table 2, page 63). Thus, the frequencies determined for rabbits (12.8%) and pigs (11%) in Hammer-1985, as well as the frequency obtained in the rats described in Mullins-1990 (62.5%), are well within the range of those observed for transgenic mice.

Applicants further submit herewith the first paper published on the use of the *tet* system in transgenic animals, namely Furth *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9302 (Appendix O). While no numbers are given for the frequency of transgenic animals obtained, the results described in Furth demonstrate that six double transgenic mouse lines carrying both regulatory elements of the *tet* system, *i.e.,* a *tet* operator linked gene of interest and a TetR fusion protein comprising a transcriptional activator, were obtained which clearly show the expected phenotype, *i.e.,* β-galactosidase activity. To date, more than 150 mouse lines are available which carry the *tet* regulatory elements that allow precise control of a gene of interest using the *tet* gene regulatory system like that of the claimed invention. A summary of these lines is available in Berger and Bujard (2004) Novel Mouse Models in Biomedical Research: The Power of Dissecting pathways by Quantitative Control of Gene Activities. In: Handbook of Experimental Pharmacology, Vol. 159. Editors: S.Offermanns and L.Hein; Springer Verlag Berlin Heidelberg. Applicants therefore submit that at the priority date of the instant application (June 14, 1993) it was possible to obtain transgenic animals other than mice with detectable gene expression and a predicted phenotype, as methods for generating and screening transgenic animals were well established in the field of transgenics.

The Examiner questions the practice of screening as a method of identifying transgenic animals with a desired phenotype. The Examiner states, "the fact that more transgenic non-human animals would need to be created before one with the desired phenotype is found clearly suggests that the phenotype resulting from transgene expression is unpredictable." Applicants respectfully disagree. Applicants have previously stated that it is routine in the art of transgenic animals to initially screen the transgenic animals for detectable gene expression and a potential resulting phenotype. Applicants also teach such practice in the working examples in the specification, wherein transgenic founder mice were identified using polymerase chain reaction (PCR) and

Southern hybridization to detect the presence of the tTA transgene or the $P_{hCMV*-1}$ luc transgene in chromosomal DNA of the mice (see page 50, lines 32 to page 51, line 14). Applicants submit that one of ordinary skill in the art will reliably identify an animal(s) who can be used to establish the future line of transgenic animals. This transgenic animal line can then be used for further discovery purposes regarding a resulting phenotype. Aside from the teachings of the specification, Applicants have provided numerous references which describe an initial screening process by which a founder is identified. Applicants maintain that the phenotype resulting from expression of the gene of interest is part of the scientific process, wherein the phenotype is often the result which confirms or contradicts the initial hypothesis.

In view of the teachings in the specification and the general knowledge in the art, the specification has provided sufficient guidance to the ordinarily skilled artisan for making and using the invention without undue experimentation. Accordingly, the specification meets the enablement requirement and Applicants respectfully request that the rejection of claims 23-40 under U.S.C. § 112 first paragraph, be withdrawn.

The Examiner has also rejected claims 27-30, alleging that only mouse embryonic stem (ES) cells were available at the time of filing. Applicants respectfully traverse this rejection. Applicants maintain that the use of homologous recombination for site specific transgene integration had been successfully demonstrated in a number of organisms at the time instant application's priority date, as described in the specification and in the references submitted in the response filed April 1, 2003. In the interest of expediting prosecution, however, Applicants have cancelled claims 27-30, thus rendering the rejection moot.

Rejection of Claims 23-40 Under the Judicially Created Doctrine of Obviousness-Type Double-Patenting

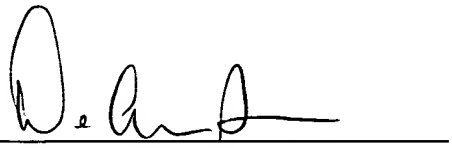
Claims 23-40 have been rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-20 of U.S. Patent No. 5,859,310. As discussed in Applicants' previous response of January 8, 2004, Applicants will submit an executed terminal disclaimer upon indication that the pending claims are allowable.

CONCLUSION

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' attorney at (617) 227-7400.

Respectfully submitted,
LAHIVE & COCKFIELD, LLP



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